PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

The Pigments of the Pieridae: A Contribution to the Study of Excretory Substances Which Function in Ornament

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Phil. Trans. R. Soc. Lond. B 1895 **186**, 661-682
doi: 10.1098/rstb.1895.0015

XV. *The Pigments of the Pieridæ: a Contribution to the Study of Excretory Substances which Function in Ornament.*

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Communicated by Professor E. Ray Lankester, *F.R.S.*

Received October 5,—Read November 15, 1894,—Revised July, 1895.

Various observations have been made during recent years, which show that a close association may obtain between pigments which function in ornament and substances of excretory nature.* Leydig† has shown that, in snakes, certain white and yellowish patches owe their colour to what he considers to be a combination of guanin with a proteid base. In the skin of *Chrysochloris* he has indicated the probable presence of urates, and has found "guanin-haltiges pigment" in the iris of vertebrates. He has further demonstrated the presence of excretory products in the epidermis of *Syrphus*, *Ascellus*, and of certain slugs. Eisig‡ has described the association of pigment with excretory substances in the Capitellidæ. Finally, Cunningham and MacMunn have extended the original observation of Barreswil§ and Voit,‖ who demonstrated the presence of guanin in the scales of fishes, and have fully dealt with the interesting association of this substance with lipochromes and melanins in the same situation.¶

In 1889, I myself showed that the pigment contained in the wings of the yellow Pieridæ was a derivative of uric acid, and suggested that it was related to the substance known as mycomelic acid.** A. B. Griffiths†† has since stated that a green pigment found by him in various species of Lepidoptera is also a uric acid derivative. In the present paper I hope to show more fully that in the Pieridæ the

* References to the literature bearing on the subject will be found in the two following papers:—H. E. Durham, "On Wandering Cells in Echinoderms" ('Quart. Journ. Micros. Sc.,' new series, vol. 33, p. 118); List, J. H., "Ueber die Herkunft des Pigments in d. Oberhaut" ('Biol. Centralb.,' heft 1, 1890).

† "Die Pigmente der Hautdecke und der Iris" ('Verhandl. d. Phys. Med. Gesellsch. Wurzburg,' bd. 22, 1888).

‡ 'Fauna u. Flora d. Golfes v. Neapel.,' vol. 16, 1887.

§ 'Comptes Rendus,' vol. 53, p. 246, 1861.

‖ 'Zeitsch. f. Wiss. Zool.,' vol. 15.

¶ 'Phil. Trans.,' vol. 184, Pt. II., p. 765, 1893.

** 'Proc. Chem. Soc.,' vol. 5, p. 117, 1889.

†† 'Comptes Rendus,' 115, p. 958, 1892; *vide*, however, foot-note on p. 680.

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normal excretory products of the insects are, throughout the group, made to subserve the purposes of ornament. The artificial production of the yellow and most important pigment will be discussed, and my original suggestion that it would be found related to mycomelic acid confirmed—to the extent that a proof will be given of its presence in the product described by HLASIWETZ under that name.

I. *The Coloration of the Pieridæ.*

The Pieridæ form a large and important group of butterflies, of wide distribution, and of various habit and food plant.

The typical colour-scheme of the group is built up of white, yellow, and black; the ground work of the wings being white or yellow, with lines, bars, spots, or mottlings of black. The black is absent in many genera, and the yellow may intensify through orange to red, though the latter colour is of comparative rarity. True blues and greens are practically absent. An important point to note is the easy interchangeability between white and yellow or orange, as shown by the colouring of closely-allied species, by that of the different sexes of the same species, and even in variations of individuals of the same sex and species.

The colours are due to the following causes :—

1. Purely optical effects, due to the minute structure and arrangement of the scales. These are of less importance in the Pieridæ than in some other groups of butterflies, and chiefly serve to modify co-existing pigmentary effects.

2. Pigments found in the scales. These may be grouped in three classes—a white pigment, pigments varying from pale yellow to orange or red, and black or melanic pigments. They are related to the scale in two different ways.

> (*a*.) They may be so intimately united with the chitin of the scale that they dissolve with great difficulty, or not at all, in solvents which do not act upon the chitin. This condition could seem to be characteristic of most of the melanic and darker pigments.
>
> (*b*.) They are found, in isolated masses, between the two layers of chitin of which the scale is made up. This arrangement is especially characteristic of the brighter coloured pigments. In the Pieridæ when the yellow pigment is extracted with hot water, as described below, the scales swell up by osmosis, the substance exudes through their walls, and, on drying, the scales return to their original shape in a colourless transparent condition.

The difference between (*a*) and (*b*) can be frequently observed microscopically. If in, say, *Colias edusa*, a scale from the black border of the wing be compared with one from the yellow region, it will be seen that the former, though darker, is more uniformly transparent than the latter. In the yellow scale the striation is more or less obscured by irregularly distributed pigment, and only when this has been extracted

are the striæ as clear as in the melanic scale, the chitin of which seems merely stained with its pigment.

3. Pigments existing, not in the scales, but between the two chitinous laminæ, of which the wing itself is composed. These, I believe, have never been described before in the adult wing.* They are found in the Pieridæ, sometimes merely modifying somewhat the ground colour of the wing (*vide infra*); but, at least in one genus, showing through the transparent chitin of the wing and also through a covering of colourless scales to function as the actual colouring matter of the wing. They have probably a morphological significance different from the scale pigments. This question will be further discussed in a later section.

II. *The White "Pigment."*

It has been frequently stated that white animal pigments do not exist. It is certain, however, that the scales of many white Lepidoptera contain substances which give opacity and "body" to the more transparent whiteness which the chitinous structures otherwise possess. Whether these are to be dignified with the name of pigments is perhaps a matter of words. The wing of the common Cabbage Butterfly, for instance, when the scale contents are extracted, loses its characteristic opaque, chalky-white colour, and shows rather the appearance common to translucent structures containing air. In this and in other white Pieridæ the substance which lends this opaque character to the chitinous scale is, I shall show, none other than uric acid.

If the wings of the common English Cabbage Butterfly or "Garden White" (*Pieris brassicæ*) be carefully removed from the body of the insect, and then extracted with dilute alkaline solutions, the extract, after filtration, will yield, on acidification, a copious white precipitate. This, after filtering and washing, will be found to give a characteristic murexide reaction. The same result may be obtained, though, of course, with more trouble, by extracting the scales after removing them from the wing membranes.

The precipitate thus obtained will be more or less amorphous. It contains impurities which appear to interfere with crystallization. To obtain a pure product, the following process may be adopted: The wings are first extracted with alcohol in the cold, and afterwards allowed to stand in cold water. The latter removes a green pigment, which is one of the class I have described above as existing between the membranes of the wing and not in the scales. It is present in very small quantity, but yields to cold water a bright chlorophyll-green solution. It will be further referred to below. The wings are now extracted once with boiling water to remove

* Dr. URECH has described a green pigment which he extracted from the unexpanded pupal wing of *Brassicæ*, 'Zool. Anzeig.,' 15, 1892, p. 281–3. This is, doubtless, the green substance referred to in Section vi.

the small quantity of the yellow pigment which is found on the under surface of the wings of *Pieris brassicæ* (and in so many other of the white Pierids). They are then boiled with dilute aqueous ammonia, or weak solutions of sodium carbonate, and the extract filtered. The alkaline extract will be always more or less coloured, in part, I think, by a pigment extracted from the black patches of the wings, but chiefly from the action of the alkali on some chromogenic substance present in the white scales. On acidification, a heavy precipitate comes down. The precipitate is pigmented, however, and must be further purified before characteristic crystals or accurate analytical results can be obtained. For this purpose it is dissolved in carbonate of sodium solution and boiled with a minimal quantity of purified animal charcoal. The colourless solution obtained after filtering off the charcoal will now yield, on acidification, a precipitate consisting of white microscopic rhombic crystals. The pure substance thus obtained gives a fine murexide reaction. It is soluble in strong sulphuric acid without decomposition, being re-precipitated on dilution. If dissolved in ammonia, it is fully precipitated on saturation with crystals of ammonium chloride, exactly as I have shown to be the case with uric acid.*

Something like a milligramme of the fully purified product can be obtained from each insect. The amount of uric acid present is about the same as that of the yellow pigment in a coloured wing of equal size. The smallness of the quantity will not seem surprising when it is stated that the whole of the scales (chitin and contents) on the wings of a medium-sized Pierid weigh less than 4 milligms. The desiccated insect itself weighs but some 60 milligms.

It is easy to obtain a murexide reaction from a single wing, but one had to employ some thousands of insects before the nature of the product could be proved.

After thorough purification good figures can be obtained on analysis; but an impurity, which raises the carbon-content, clings with some obstinacy to the product. Analyses should not be made till the substance will dissolve in strong sulphuric acid, without charring.

	C.	H.	N.
Pieris brassicæ.			
A pure white product gave	35·90	3·21	
A product which H_2SO_4 charred slightly, gave	..	..	32·91
†*Pieris rapæ* and *brassicæ* (mixed)	36·46	3·01	
Pieris canidia (Chinese species)	..	..	32·65
Calculated for uric acid	35·71	2·38	33·33

There can be no doubt that a murexide-yielding substance, having the properties described, and giving the above results on analysis, is uric acid. But there are some

* 'Proc. Roy. Soc.,' vol. 52, p. 93, 1892.

† Pure uric acid, burnt in same tube as above, 35·80 C, 3·02 H.

peculiarities about its crystallization which might lead an observer, working on a small scale, and with qualitative methods only, to feel some doubt on the matter, in spite of the ready murexide test which he will obtain. In general, impure uric acid will crystallize more readily than will the pure substance itself. But, in the present case, as I have said, the original precipitate obtained from extracts of the wings remains, with some obstinacy, amorphous. After purification the crystals become characteristic enough, taking the forms proper to pure uric acid, and not those of urine sediments. The commonest forms are rhombic prisms with truncated angles, and these tend to form rosettes. In solutions of intermediate purity the crystals may chiefly comprise rosettes of fine needles, but approximations to the whetstone form of urine sediments are not uncommon.

Although the contents of the white scales are to all appearance amorphous, I am inclined to think that the acid exists in them mainly uncombined, and not as urates. If it be combined with a base it is certainly in a form analogous to the quadrurates, because, although the amorphous tendencies of the extracted substance as described are always in evidence, it is quite certain that characteristic crystals of the free acid may eventually be obtained in aqueous extracts of the wings without any acid being added. Such extracts are, in fact, markedly acid themselves while hot. I have not yet had a supply of material large enough to spare any for the proper investigation of possibly combined bases, but the point is of no great importance at present. The histological relationship of the uric acid to the scale is exactly that of the yellow pigment found in allied insects, or existing in other scales on the same wing, that is to say, it is found as a deposit between the chitinous layers of which the scale is made up.

III. *The Yellow Pigment.*

It will be shown later that the same substance functions throughout the group wherever yellow or orange appears in the colourings of any species. But the pigment of the common English Brimstone Butterfly (*Gonepteryx rhamni*) is here dealt with as an example.

If the wings of male insects of this species, after removal from the bodies, are first extracted successively with alcohol and with cold water, and are afterwards heated with distilled water, the yellow pigment dissolves in the latter with the greatest ease. On filtering and cooling, an amorphous yellow powder separates, which may be filtered off and dried. This will be found to exhibit the following properties. It is quite insoluble in alcohol, ether, chloroform, and all ordinary organic solvents. It is practically insoluble in cold water, but dissolves with great readiness on heating, the solution being bright yellow, and markedly acid to litmus. The hot solution fluoresces with a fine green colour, the fluorescence being especially well marked at the surface. The immediate and complete precipitation of the pigment which occurs on cooling its hot aqueous solutions is highly characteristic.

The substance is easily soluble in cold alkaline solutions, and is re-precipitated therefrom on acidification. Its solutions in ammonia are more fluorescent than aqueous ones, and even very dilute solutions fluoresce powerfully after the addition of zinc chloride, or cadmium iodide, and ammonia. In very weak solutions the fluorescence is blue.

It yields no definite banded spectrum, but only a diffuse absorption of the violet.

Although remaining amorphous under all circumstances, the pigment is perfectly well characterised in its physical properties, never showing the least tendency to form those gummy or syrupy residues which are apt to be rejected of the chemist. However prepared, it is, when dry, an orange powder, not unlike oxide of iron in appearance, and neither deliquescing nor altering in any way when exposed to the air. It is precipitated from its solutions by most of the heavy metals. It dissolves in strong nitric acid with decomposition, and the solution yields murexide on evaporation. A fine murexide reaction may be obtained from the hot aqueous extract of a single wing; but the result is improved if the pigment be purified by first extracting the wing with alcohol and then with cold water. The identity of the murexide obtained may be confirmed by means of its spectrum. One finds that murexide solutions yield definite bands only in strengths of very limited range. But if the red product from the yellow pigment be compared side by side with murexide prepared from uric acid, they will be seen to be identical.

By far the most important reaction yielded by the yellow pigment is seen when it is heated in open vessels on the water-bath with dilute mineral acids (15 to 20 per cent. sulphuric). Under these circumstances it is slowly but continuously converted into a purple substance, which yields a definite, characteristic, and easily recognized spectrum. For the sake of convenience, I shall speak of this substance as "lepidoporphyrin"—a name which justifies itself by its analogies.

The purple derivative is sharply distinguished from the original pigment by its complete insolubility in hot water. Thus, if a quantity of the yellow pigment be dissolved in hot water, to which about 15 per cent. of sulphuric acid has been added, and if the solution be then heated for an hour or two over the water-bath, water being added from time to time to prevent over-concentration, on cooling, a precipitate will come down consisting of the purple substance mixed with some unaltered yellow pigment. The mixture being filtered off and washed free from acid, the yellow body may now be wholly removed by treatment with boiling water, in which the purple substance is quite insoluble. The spectrum of the latter (to be presently described) can be observed, however, in a solution of the yellow body to which acid has been added, very shortly after heating has been commenced. The change goes on slowly in the cold in the presence of free mineral acids.

The conversion is apparently complete. It is aided, however, by separating the purple body from time to time and continuing the process in the filtrate.

Properties of "Lepidoporphyrin."—In its general properties the substance would

seem to have no analogy whatever with murexide and the purpuric acid compounds. When separated from the parent yellow pigment in the manner described, it is an amorphous powder of fine purple colour. It is insoluble in water, hot or cold, but more soluble in the presence of excess of mineral acids. It is insoluble in alcohol, ether, and other organic solvents.

In alkalies it dissolves without change in its spectrum; but, after solution, it is decomposed by them with some rapidity.

It is soluble without decomposition in strong sulphuric acid, to which it imparts a fine purple-red colour; such solutions in oil of vitriol are quite permanent and may be kept indefinitely. On full dilution of the acid, and especially after partial neutralisation of the solution, the substance separates completely in reddish-purple, transparent, gelatinous flakes. These may be re-dissolved in strong acid and re-precipitated, and the process may be repeated indefinitely without the least change in the properties or spectrum of the substance. In this respect it much resembles hæmatoporphyrin.

Spectrum of "Lepidoporphyrin."—In acid solutions this is, as I have said, well marked and characteristic. It consists of two absorption bands; one in the green, between the lines D and E, and another, somewhat broader, near F.

The following are the wave-length measurements observed in products obtained from various species of Pieridæ. In each case the purple substance was dissolved in strong sulphuric acid:—

Gonepteryx rhamni	(*a*) λ 560 — λ 532,	(*b*) λ 515 — λ 493,
Colias edusa	(*a*) λ 560 — λ 530,	(*b*) λ 517 — λ 491,
Euchloë cardamines . . .	(*a*) λ 558 — λ 530,	(*b*) λ 515 — λ 493,
Callidryas argante	(*a*) λ 560 — λ 530,	(*b*) λ 518 — λ 493,
Delias eucharis	(*a*) λ 558 — λ 530,	(*b*) λ 515 — λ 493.

The two bands thus observed are very definite, and seem to vary scarcely at all in breadth in solutions of different strengths. The bands seen in alkaline solutions agree in position with those just given, but, as already stated, the substance is quickly decomposed by alkalies, the solutions losing their colour and the bands disappearing. It may be here noted that carminic acid dissolved in sulphuric acid yields two absorption bands, the centres of which agree exactly in position with those of the lepidoporphyrin bands, but the action of alkalies sharply distinguishes the two substances. I have as yet been unable to prepare a sufficiency of lepidoporphyrin for the purpose of analysis. It contains about 30 per cent. of nitrogen.

IV. *An Artificial Product probably identical with the Yellow Pigment.*

In 1853 Wöhler* described certain observations on the effects of heating uric acid with water in sealed tubes at high temperatures. The tubes, originally filled

* 'Ann. Chem. Pharm.,' ciii., p. 118. An editorial note signed "W."

with suitable quantities of the acid and distilled water, were found, after exposure for eight days to temperatures varying from 100° C. to 140° C., to contain a yellow fluorescent solution, which, upon cooling, set into a gelatinous mass. "This," to quote WÖHLER, "consists of acid ammonium urate, coloured by a new-formed body, which, however, is to be obtained only in very small quantity." Almost simultaneously with the publication of these observations, HLASIWETZ* described experiments made on lines which were in all essentials identical. The latter chemist, however, considered the yellow gelatinous substance which separated from the tubes to be a chemical individual, and he identified it with the compound previously described by LIEBIG and WÖHLER under the name of mycomelic acid. This conclusion has been generally accepted, and, in the text-books, heating uric acid with water will be found described as one of the methods of preparing mycomelic acid.

I think it is easy to show that HLASIWETZ was mistaken in the inferences he drew from his experiments, and that, as a matter of fact, the real yellow product of the reaction is formed in minute quantity only, merely pigmenting a residue of unaltered ammonium urate as stated by WÖHLER. I have many times carefully repeated the operation exactly as described by HLASIWETZ, and I have also varied the conditions of the process in every conceivable way, but under no circumstances does it seem possible to obtain more than a minute yield of the yellow substance. In spite of the small yield, however, it is possible, as will be later seen, to adduce evidence to show that this artificial derivative of uric acid is closely related to, and, in all probability, identical with the natural yellow pigment described in the last section.

In perusal of the original paper by HLASIWETZ, one notes at the outset that the author entirely ignored the presence of ammonia in his tubes, though, at the temperature he employed (190° C.), ammonium carbonate is formed in quantity before the yellow product has appeared at all. His procedure was to concentrate the solution direct, and to filter off "the yellow, flocky, gelatinous, uncrystallizable substance," which separated on cooling. It was this product he analysed. Now, if the figures given by his analyses be examined, they will be seen to agree closely with those proper to ammonium urate, whereas they are only made to agree with the composition usually assigned to mycomelic acid by the purely gratuitous assumption of a retained half molecule of water. As a matter of fact, the aqueous solution of a yellow product, prepared exactly in accordance with the directions given by HLASIWETZ (*loc. cit.*), will be found, if boiled up with animal charcoal and filtered, to yield a colourless filtrate, from which, on acidification, uric acid crystals separate in quite colourless condition. The weight of these, calculated to ammonium urate, will be found to represent a very large fraction of the total yellow substance taken to boil with charcoal. It is, however, not wholly surprising that HLASIWETZ was led to look upon his product (which was obtained at temperatures higher than those used

* 'Ann. Chem. Pharm.,' ciii., p. 211.

by Wöhler) as a chemical individual. The association between the urate and the yellow product is extremely close. I have found no solvent that will separate them, and no amount of fractional precipitation appears to have any effect in this direction. If, moreover, the yellow solution from the tubes be acidified, the uric acid thus precipitated is just as closely associated with the coloured substance. It separates as an amorphous yellow powder and remains obstinately amorphous, even if dissolved and re-precipitated an indefinite number of times. This association with the yellow substance entirely prevents crystallization of the uric acid and greatly increases its solubility in hot water. The figures yielded by analysis of the yellow mixture scarcely differ however from those given by pure uric acid, and boiling with charcoal will, as before, yield a colourless crystalline product. It cannot be doubted, in fact, that the "mycomelic acid" of Hlasiwetz was but pigmented ammonium urate. The results are practically the same through wide ranges of temperature. The yellow substance is but a bye-product in the general process of hydrolysis which goes on in the tubes, appearing at a stage in the process which varies somewhat with the temperature employed. But the great difficulty is that, as soon as formed, it shares itself in the hydrolysis, and no adjustment of the conditions will prevent this. The consequent smallness of the yield, and the obstinately close union with unaltered urates, make any attempt to purify the yellow substance extremely difficult.

The substance, however, is one of very high tinctorial power, and solutions of pigmented urate from the tubes are of fine yellow colour and strongly fluorescent. The colour and fluorescence of such solutions are indeed identical with those of the natural yellow pigment, and are affected in identical manner by reagents. So close is this resemblance that, with the knowledge that both are related to uric acid, one cannot but be impressed with the likelihood of the identity of the yellow substances.

More direct evidence of the identity thus suggested is found in the fact that the artificial product under treatment similar to that which converts the natural pigment into lepidoporphyrin yields a purple derivative, apparently identical with the latter substance.

A yellow solution of pigmented urate, prepared according to the method of Hlasiwetz, will, if heated on the water-bath with 15 per cent. sulphuric acid, gradually come to show the absorption bands of lepidoporphyrin, and will eventually yield a purple precipitate of this substance. But the reaction is clogged by the close association of the large proportion of uric acid with the yellow substance, and may be slow to commence. A modified procedure, however, enables the artificial product to be obtained with greater ease. This consists in adding to the original mixture of uric acid and water, before heating, sufficient sulphuric acid to neutralize all the ammonium carbonate, which is else formed in the tubes. Hydrolysis is then accomplished in the acid condition without much change in the ultimate yield of the yellow bye-product. The tubes, when removed from the furnace, have, it is true, a lighter colour than when pure water is used, but merely because alkaline solutions of the yellow substance

are darker than corresponding acid ones; the actual quantity formed is much the same in both cases. But the circumstance that in the acidified tubes much less unhydrolized uric acid goes into solution prevents in some measure its troublesome association with the yellow substance in the final product; something like a separation is to be obtained, and from such products artificial lepidoporphyrin may be prepared with ease.

It will be best to describe at this stage an actual experiment in which the purple derivative was successfully obtained. Six tubes, each containing 2 grms. uric acid, 20 cub. centims. water, and 5 cub. centims. strong sulphuric acid, were sealed and heated for three hours at 190° to 195° C. After cooling they were opened, the mixed contents transferred to a beaker, diluted with water and heated to boiling. The solution was filtered hot, yielding a yellow fluorescent filtrate, which on cooling deposited a yellow amorphous precipitate. This was filtered off, washed with cold water, and dried in the air bath; the dried substance was then boiled up with 100 cub. centims. of dilute sulphuric acid (15 per cent.), the solution filtered, and heated on the open water-bath. The bands of lepidoporphyrin could be seen in this solution after the lapse of about an hour. It was heated for three hours, the bulk of solution being maintained by occasional additions of water, and finally allowed to stand for 24 hours. The precipitate, which had then settled, contained a large portion of the purple substance. It was dissolved in strong sulphuric acid, yielding a rich carmine-red solution, which was examined spectroscopically. It showed bands identical with those given by lepidoporphyrin. The following measurements were obtained with a spectroscope belonging to Dr. A. E. Garrod, who kindly checked the readings:—

Artificial lepidoporphyrin . . . . . (*a*) λ 560–λ 532, (*b*) λ 515–λ 494,

Lepidoporphyrin from *Gonepteryx rhamni* . (*a*) λ 560–λ 532, (*b*) λ 515–λ 493.

A large number of preparations of the artificial substance have given identical results.

As might be expected, the impure products from the sealed tubes never give lepidoporphyrin with the ease and rapidity found in the case of the natural pigment, but, if the right conditions are observed, they never fail to give a plentiful yield. For some reason (probably because a further separation from uric acid is obtained on subsequent extraction) the change takes place more rapidly if the tube products are dried, as described above, before the treatment with sulphuric acid on the open water-bath. No purple, it should be observed, is ever pre-formed in the closed tubes, even when acid is present. Lastly, it may be noted that in the spectrum of artificial products a narrow band may occasionally be seen to the red side of, and close up to, D. This belongs to a more soluble body, which will indeed dissolve in water. On boiling with dilute sulphuric acid, this is entirely converted into lepidoporphyrin.

In its solubility and behaviour towards re-agents, the artificial lepidoporphyrin agrees exactly with that obtained from the natural pigment. Anyone wishing to prove the identity of the two products, may do so with the greatest ease on quite a small

scale. A grm. of uric acid, heated with acidified water in a sealed tube, and the wing-extracts of a couple of Pierids, will each yield ample lepidoporphyrin for the purposes of identification and comparison.

I think it best to make no claim for having obtained proof of identity between the natural and artificial yellow substances beyond what is given by the qualitative evidence just detailed. Nevertheless, after hydrolizing in the acid condition large quantities of uric acid—half a kilo and upwards being heated in an autoclave—I have fractionated out, by repeated solution in hot water (drying the extract before each fresh treatment) products which yield lepidoporphyrin with great ease, and at the same time give figures on analysis which agree closely with those to be detailed for the pigment in the next section. But the conditions are difficult to define, and I hesitate to describe the method as one by which a pure substance can be prepared with any certainty. I have now become the less concerned to prepare such products because, while the process by which they are obtained—hydrolysis at high temperature—is not one likely to throw much light upon the constitution of the pigment from a synthetical point of view, repeated efforts have convinced me that the yield must always be so small, that for analytical work it is nearly, if not quite, as convenient to work with the natural substance.

V. *Analysis of the Natural Yellow Pigment.*

Having regard to the fact that the substance is an amorphous one, its purity being therefore difficult to demonstrate, the following analyses are given with all due reserve. But it will be seen that substantial agreement is obtained in the figures yielded by pigment from entirely different species, and even from different genera, a fact which should certainly add to their value. A sufficient number of a given species of insect to yield pigment for quantitative work is seldom to be obtained on any one occasion. The analyses were therefore made at long intervals during a period of more than three years, the combustion apparatus used varying in different experiments. All the results obtained are given below, no kind of selection of more concordant figures having been made.

In all cases, the wings, after removal from the bodies of the insects, were allowed to stand for two or three days in rectified spirit. This was then replaced by cold water, and the wings again allowed to stand for a day or two. Considerable quantities of soluble constituents are thus removed. The pigment was then extracted by heating with distilled water. The hot yellow aqueous extract was filtered and cooled, when the pigment separated out almost completely. It was filtered off, washed with cold water, and dried in glass basins at 100°. The residue was then extracted twice or thrice in the basin with boiling absolute alcohol, and finally dried at 110°. Any further treatment employed is given before the figures of the analysis in each case.

I. *Gonepteryx rhamni* (male).—500 insects yielded about ·350 grm. of pure pigment, obtained by dissolving the original product, prepared as above described, in ammonia and re-precipitating with acetic acid. The washed precipitate was dried at 110°.

·1748 grm. gave CO_2 ·2457, and H_2O ·0497. C = 38·33 ; H = 3·16.
·0746 grm. gave 22·7 cub. centims. moist N at 758 millims. and 3°. N = 37·70.

II. *Colias fieldii.*—120 insects yielded ·0935 grm. purified as in I.

·0598 grm. (dried 110°) gave ·0829 CO_2 and ·0194 H_2O.
C = 37·81 ; H = 3·60.

III. *Terias lisa.*—200 insects yielded about ·100 grm. only. Pigment dissolved in Na_2CO_3 and re-precipitated by acetic acid.

·094 grm. (dried 110°) gave ·1305 CO_2 and ·0288 H_2O.
C = 37·86 ; H = 3·40.

These three separate samples of pigment from entirely different species show such substantial agreement (although the *Colias* analysis was on very small quantities), that it afterwards seemed justifiable to work on mixed species.

IV. *Gonepteryx rhamni* (245 male insects) mixed with various species of *Terias* (204 insects). Yield about ·320 grm. after purification as in I.

·1090 grm. gave CO_2 ·1521 and H_2O ·0330. C = 37·97 ; H = 3·35.
·0956 grm. gave CO_2 ·1346 and H_2O ·030. C = 38·40 ; H = 3·48.
·0489 grm. gave 15·8 cub. centims. N. at 768·5 millims. and 19·5°. N = 37·47.

V. *Gonepteryx rhamni* (120 male insects). Yield about ·90 grm. after purification, as in I.

·0826 grm. (dried at 120°) gave CO_2 = ·1149 ; H_2O ·0253.
C = 37·94 ; H = 3·44.

VI. *Colias edusa*, purified as in III.

·0528 grm. (dried at 110°) gave 16·8 cub. centims. N. at 760 millims. and 21°·5.
N = 36·15.

Summary.

	C.	N.	H.	O.
Gonepteryx rhamni	38·33	37·70	3·16	20·81
	37·94	..	3·44	..
Gonepteryx rhamni and *Terias*	38·40	37·47	3·48	20·65
	37·97	..	3·35	21·21
Terias lisa	37·86	..	3·80	..
Colias fieldii	37·81	..	3·60	..
Colias edusa	..	36·15	..	..
Mean	38·13	37·11	3·47	21·29

VII. *Colias edusa.*—In the following case the pigment, after initial purification, was precipitated as a silver-salt, and the latter decomposed by warming with ammonium chloride. The ammonia compound obtained was then decomposed by means of sulphuric acid, and the pigment separated from traces of silver chloride by re-solution in hot water. The quantity left for analysis was too small for accuracy, but I give the figures to complete the series :—

·0478 gram. gave ·068 CO_2 and ·018 H_2O,
·0269 gram. gave 8·2 cub. centims. N at 770·4 millims. and 22°,
C = 38·80 H = 4·20 N = 38·13.

We thus see that the acid pigment, when separated from possible traces of combined bases, is in all probability identical in various species which differ rather widely in colouring and habits. This will be later seen to receive confirmation from analysis of the silver compounds. *Gonepteryx rhamni* and the various species of *Terias* used for the above analysis are a pale sulphur-yellow in colour, while *Colias fieldii* and *edusa* are of full orange colour. To some extent, though not in a marked degree, the extracted pigment keeps the distinction found in the wings as regards depth of tint, as though the difference were one of molecular condition. Still, the actual shade of the purified product varies with the mode of preparation.

Silver Compounds of the Yellow Pigment.—Hot aqueous solutions of the pigment yield on the addition of silver nitrate an orange gelatinous precipitate which shows no tendency to reduce even on boiling. In order to secure complete precipitation, the substance which, as already stated, is markedly acidic in its character, should be first exactly neutralized ; otherwise very dilute solution must be employed. It is very difficult to dry the silver compound completely without the occurrence of some decomposition, and experience has finally led me to adopt the following plan for the determination of the percentage of silver. A weighed quantity of the purified

pigment is dissolved in ammonium hydrate, and the solution evaporated to dryness on the water-bath to remove excess of ammonia. The residue is then dissolved in water, and the solution ascertained to be exactly neutral to litmus. Such a solution is completely precipitated by silver nitrate, the filtrate from the precipitate being absolutely colourless. The silver may be then determined and the percentage calculated from the weight of pigment originally taken, without the necessity of drying and weighing the silver compound. By this method the second, third, and fourth of the following results were obtained, the first by precipitation of the weighed pigment in hot aqueous solution largely diluted (800 cub. centims.). In each case the precipitate was dried and ignited, the silver weighed, and the result checked by titration by VOLHARD'S method.

Species.	Weight of pigment taken.	Silver titrated.	Indicated molecular weight.
	mgms.	mgms.	
Gonepteryx rhamni	100	71·5	150·9
" "	250	178·4	151·2
Colias edusa	145	103·2	151·6
Callidryas (mixed species) . .	96	68·0	152·3

The *Gonepteryx* pigment which gave the first result was prepared from insects which had been some years in a cabinet. In the second case the insects were bred during the present season (1895) and killed within a few hours of emergence from the pupa.

If the precipitation by silver is carried out in the presence of excess of ammonium hydrate a compound is obtained which contains ammonia and a larger percentage of silver (50·7 per cent.). It would seem to be a molecular compound of the formula Ag_2X, $Ag\ NH_4X$, in which the substance acts as a dibasic acid; but it is referred to here only to emphasize the fact that excess of alkali should be avoided in preparing solutions for precipitation as described above.

It would serve no good purpose to attempt, with the above evidence, to assign a definite formula to the yellow pigment, but the following points are, I think, worthy of consideration. As I have ventured to assert above, the fact that substantial agreement is seen in the analyses of products from insects of divers species, the locality, habits, and food plants of which differ widely, is strong evidence that a chemical individual is being dealt with, in a condition of, at least, approximate purity. This is certainly borne out by the general behaviour of the product as prepared for analysis, for, after purification as described above, it permits of no sort of fractionation that I have been able to discover.* The simplicity and completeness of the reaction by which it is converted into lepidoporphyrin is further evidence in the

* Experience with HLASIWETZ product naturally led one to suspect that the wings might contain an analogous mixture, and a large proportion of the available material was spent in a wholly unsuccessful endeavour to effect a separation.

same direction. If it be admitted that the substance is a chemical individual, the composition of its silver compound shows clearly that its molecule is of the same order of magnitude as that of uric acid itself, and is not of the complexity which might be expected in a pigmentary substance. As I have stated, the action of dilute mineral acids (such as sulphuric and hydrochloric) upon it, is solely to produce lepidoporphyrin, and the latter substance yields no murexide reaction. But, if the yellow pigment be carefully oxidised with warm dilute nitric acid, no more of the reagent being used than is sufficient to decolorize the solution, colourless crystals of uric acid may be obtained on cooling. From the above facts it would seem to be at least probable that the difference between uric acid and the pigment is one of oxidation. That the relation between them is a simple one is further suggested by biological evidence.

Slight climatic influences may so affect metabolism in the pupa that yellow takes the place of white in the wings. Thus, the common English white butterfly, *P. rapæ*, which I have used for many of my experiments, after migrating to North America, takes on an uniform yellow flush,* and the pigment of this variety I can show to yield plentiful lepidoporphyrin. Such a variation, it seems to me, one might expect to be a question of oxidation.

The figures of the above analysis will be found to agree not unsatisfactorily with the composition of a substance derived from uric acid, by the replacement of an atom of oxygen by two of hydrogen, and the molecular weight required for this (154) is in fair agreement with what is indicated by the silver salts. It is, perhaps, unlikely that the actual constitution of the pigment will be demonstrated until the artificial substance has been prepared from uric acid by a reaction of more determinate nature than that of hydrolysis at high temperatures.

Since the product of HLASIWETZ is certainly not mycomelic acid, there was no *à priori* likelihood of obtaining the pigment by the other reactions which are supposed to yield this latter substance. As a matter of fact, a careful investigation has convinced me that the product of the action of ammonia on alloxan (LIEBIG and WÖHLER) and the body produced by boiling azulmic acid with water (JACOBSEN and EMMERLING) are substances distinct from each other and from the HLASIWETZ product. They give no reactions which connect them with the pigment.

While waiting further evidence as to its precise nature, the yellow Pierid pigment may be conveniently known as *Lepidotic acid*.

VI. *The "Interlaminar" Pigments.*

In an early section of this paper I referred to the fact that, in addition to the scale pigments, which I have now shown to be clearly excretory in nature, there are to be found in butterflies (in the Pieridæ, at any rate), existing between the chitinous mem-

* SCUDDER'S 'Butterflies,' p. 164.

branes of which the wing is formed, pigments of different nature and significance, which may fittingly be termed interlaminar pigments.

As is well known, the chitinous framework of the wing in Lepidoptera may be easily separated, by soaking in water or other means, into two separate laminæ, and the space between these is the remains of a cavity which, in the embryo wing, is continuous with the body-cavity. In the imago, when the wings have dried after emergence from the pupa, these two laminæ are more or less adherent; but, in the insects I am referring to, they are separated by a small quantity of pigment, which, from its position, is probably related to the blood pigment. From the wings of the yellow and white Pieridæ, with which I have chiefly worked, cold water extracts a brilliant green pigment, which is easily seen, if the scales be removed, to be interlaminar in origin.*

It contains iron, and yields a spectrum with a well-marked band in the red. The spectrum is not that of chlorophyll. This substance is not of great importance in determining the wing-colour of the white and yellow insects, though it alters the shade in a slight degree. But I have been able to find at least one genus (*Nepheronia*) in the Pieridæ where a pigment of this sort is of chief functional importance in ornament. Thus, in the species *N. lutescens* (BUTL.) the males have the ground colours of the wings of a delicate blue shade, which is entirely due to an interlaminar pigment showing through a covering of colourless scales. The females of the same species show the ordinary yellow pigments. It is thus seen that in butterflies, as elsewhere, what is probably a blood pigment may function in ornament simultaneously with the epidermal pigments—a point which is of interest in association with Mr. POULTON'S classification of like phenomena in Lepidopterous larvæ.†

VII. *The General Pigmentation of the Group considered in Relation to the above Results.*

The black pigment is, as I have said, very intimately associated with the chitin of the scale, and does not appear to be extracted satisfactorily by any solvent that I have been able to employ. I have made no attempt, therefore, to deal with it in the present paper.

All other scale-coloration in Pierids is produced by means of uric acid, its yellow derivative, and, probably, by only one other allied scale-pigment to be later described. Colours not directly due to these substances are produced by super-added optical effects, or by the interlaminar substances described in the last section.

Uric acid itself occurs, I believe, in the wing-scales of all the white Pieridæ, and probably wherever a chalky-white patch is found on the wing of a Pierid. This statement is based upon the evidence of the murexide test alone, except in the case

* *Vide* foot-note, p. 663.

† 'Proc. Roy. Soc.,' vol. 38, p. 269, 1885.

of the three species which I used for quantitative work (Section III.). Of these, *Rapæ* and *Brassicæ* are closely allied, but *Canidia* is of a type so far different from the other two as to give valuable evidence of the general distribution of uric acid in the group.

The yellow pigment one can trace with the greatest ease, even when a single insect is alone available, by the recognition of the spectrum of its derivative purple body. By this means, and by the use of the murexide test, I have traced it in all the typical genera of the Pieridæ. I have employed many scores of species, and may say that, with the single exception of insects whose colouring is due to what I have termed interlaminar pigments, every coloured patch on the wing of a Pierid will be found to yield more or less lepidoporphyrin. The best method of applying the test on a small scale (say by the use of from one to three insects) is first to dip the wings in a little spirit, to allow of their being more easily wetted subsequently by water. Next to transfer them to a test-tube (of course after careful removal from the bodies), to heat them for a few minutes with water, to filter off the yellow extract into a second test-tube, and finally, having added 20 per cent. of sulphuric acid, to heat the solution for half-an-hour on the water-bath. The yellow solution gradually becomes more and more red in colour, and the absorption bands (Section III.) are soon visible. After a while the purple body separates out, and, if the supernatant fluid be now pipetted off, strong sulphuric acid will strike a magnificent red colour with the precipitate, and the bands may be seen still more plainly in the strong acid solution.

If one examines a representative collection of Pierids, it is quite evident that the chief colour-energy of the group is expended in yellow and orange. In many genera yellow is almost universal, and in all such it is easy to show that the substance described in this paper forms the chemical basis of pigmentation. The wide distribution of such a genus as *Terias*, and the multiplicity of species it comprises, as well as the magnificent coloration in the males of the larger species of *Gonepteryx*, *Amynthia*, and allied genera, give to this yellow "lepidotic acid," which colours them, a position of some importance as an animal pigment.

There is one point I have observed which is, perhaps, not easy of explanation, but which may be fitly referred to here. Uric acid and the yellow pigment are very commonly found together in different areas of the same wing; when, therefore, one had to deal with a yellow insect which has a uniform ground colour paler than normal, it seemed not unlikely that each individual scale might contain both bodies, the yellow being diluted by the white. That is to say, the change in metabolism which has led uric acid to be replaced by its yellow derivative would, in such cases, have been partial only. I have been unable, however, to get clear evidence of the presence of uric acid in these pale yellow insects, and the lighter colour seems to be due to a smaller proportion of pigments.

As an instance of this, the pale species of *Colias* may be mentioned; *C. hyale*, for example, might be thought to differ from its darker congeners in its pigment being

diluted with white, but I have not been able to separate uric acid from this species. The two substances seldom or never coexist in the same scale.

In the light coloured females of yellow genera, like *Gonepteryx* and *Amynthia*, there is also an absence of unaltered uric acid. Save for the chlorotic effect of the green interlaminar pigment so often referred to, their colour is due to the unpigmented chitin of the scales. Their appearance is that of true white species after extraction of the uric acid.

Where yellow gives place to orange, the effect is generally due merely to an increased quantity of pigment. On the other hand, it is possible that in certain cases a pure yellow may be made orange by slight admixture with the closely allied red substance now to be mentioned.

Actual red is a colour by no means common on the wings of Pierids. It is developed, however, in *Delias* and allied genera, typically in the form of marginal spots on the under surface of the hind wing.

It has proved difficult to obtain a sufficient supply of this red pigment for a proper investigation of its properties; but it is easy to show that it differs very little, chemically, from the yellow substance. It is freely soluble in hot water, its aqueous solution being yellow, exactly similar in appearance to that of the commoner pigment as obtained, for instance, from a yellow area usually found upon the same wing. It yields lepidoporphyrin with equal ease, and it gives the murexide reaction. (No lepidoporphyrin is present in the original extract from the red spots.) On one occasion I was fortunate enough to obtain a supply of a species of *Delias* (*D. eucharis*) sufficient for the preparation of its silver compound. This (precipitated in ammoniacal solution) contained 51 per cent. of metal, which will be seen to agree with the result obtained from the yellow substance under like circumstances (Section V.). In many respects, therefore, it closely resembles the yellow pigment, but its yellow aqueous extract when evaporated leaves a residue which on drying is distinctly red. Whether this betokens a mere physical difference, or whether the yellow body (which, as I have shown, is markedly acidic in character) may be here combined with some base, I am at present unable to say. It is sufficient for my present purpose to show that the pigments are closely allied; but the point is an important one for settlement nevertheless, since the yellow lepidotic acid and its red modification are practically the only coloured scale-pigments in the whole group of the Pierids. In the rare cases where colours other than yellow, orange, or the brick-red colour found in *Delias* occur, the chemical basis is still the same, modified by purely optical effects; of these I shall give instances immediately.

It is interesting to note, by comparing various allied species of *Delias*, that the red marginal spot may become more yellow, while the yellow area usually found at the root of the same wing may become more red, till both may exhibit a uniform orange colour, or the change may go farther and red and yellow change places without the general colour-plan of the wing being altered.

Cases among Pierids where the typical colours of the group are departed from, are by no means common. A green colour, found, for instance, on the under surface of the English Orange Tip (*Cardamines*) and on many allied species, is well known to be entirely due to admixture of black and yellow scales. The greenish hue of certain arctic species of *Colias* is also due to admixture of melanic scales throughout the yellow of the wing.

In a genus known as *Appias* one finds a uniform coloration of a warm reddish-brown tint, at times almost resembling the colour of mahogany. But the wings of these insects yield a solution of lepidotic acid, from which plentiful lepidoporphyrin may be obtained. After extraction with hot water the chitin of the scales remains tinted with what appears to be a very small amount of the melanic pigment, which will account for the brown, as distinct from yellow, colour of the wings.

But it is in certain members of the group usually described under the name *Teracolus*, that the greatest departure from the normal pigmentation of Pierids occurs. The orange "tips" which are common on the upper wings of this group may be replaced by a brilliant red, in which the pigment I have described in *Delias* undoubtedly is present. But the red may take a bluer shade, and finally, in such species as *T. imperator* (Butl.), *T. coliagenes* (Butl.), and others, one finds a very striking development of colour strongly suggesting the effect of aniline dyes of the magenta type. On extracting these brilliant colour patches with hot water, however, one gets a *yellow* solution from which lepidoporphyrin may be obtained with ease. The wing after extraction is practically colourless. In fact, the only pigment present is, once again, lepidotic acid or its red modification, the remarkable colour effect on the wing being due to a superadded interference effect caused by certain finely striated delicate scales, which, in the extracted wing, may be seen mingled with the ordinary scales which have contained pigment. In making this observation, I have chiefly worked with *Anthocaris zoë*, a finely coloured insect of this type, for my supply of which I was indebted to Mr. Grose-Smith. I have also employed specimens of *Imperator* obtained from the same source.

The scale-pigments of the Pieridæ are thus few in number, closely related, and easily identified. I have never found a coloured patch on any single species which would not yield a yellow solution to hot water, nor one from which the murexide and lepidoporphyrin tests could not be obtained.

VIII. *The Pigments of other Butterflies.—Pigments in Mimicry.*

Although uric acid and its soluble murexide-yielding derivatives are so universal in the Pieridæ, they seem to be largely confined to this group of Butterflies. I do not pretend to have so thoroughly explored the Lepidoptera as to be able to deny their occurrence elsewhere in this large class of insects, but I have examined species representative of all the more important sub-families among the Rhopalocera without

finding evidence of their presence.* It is in the highest degree probable that other lepidopterous pigments are allied to excretory products, but no other group offers clear evidence of this, such as is found in the Pierids.

In the group usually held to be most nearly related to the Pierids, the *Papilionidæ* proper, I have examined many species without finding allied pigments. The yellow substance found, for instance, in such an insect as the common English "Swallow-tail" has entirely different properties. It may be extracted with hot water, but it yields a gummy residue from which neither murexide nor "lepidoporphyrin" reactions can be obtained. A red pigment, common in the Papilionids, is very slightly soluble, but if treated on the wings with acids it becomes of a faint yellow colour. The red may be restored by treatment with alkalies, or by thorough washing with cold water till the acid is removed. This reaction was, I believe, first described by Mr. F. H. Perry-Coste.† This pigment may be well studied in the species *Papilio* (*vel Mene laides*) *aristolöchæ*, where the action of acids on the red spots produces an appearance almost exactly like that of the females of *Laertias pammon*—insects which are supposed to mimic *Aristolochæ*, but which have yellowish instead of red spots. No murexide or lepidoporphyrin can be obtained from this substance.

Among the *Nymphalidæ* one finds many pigments which are soluble in hot water or dilute alkalies, but none of these, so far as I have been able to discover, yield the characteristic reactions of the Pierid pigments. The brighter pigments, such as the reds found in the *Vanessidæ*, are the more characteristically soluble; the darker pigments being less so. In this family occurs the remarkable white pigment which turns bright yellow with alkalies. It is found in the English "Marbled White," *Arge galathea* (*vide* 'Nature,' vol. xxx., p. 571). It yields no trace of murexide. In the *Lycænidæ* chemical pigments play a subordinate part, interference colours predominating. From no species of this group have I been able to extract murexide-yielding bodies. Lastly, the brown pigments of the *Hesperiidæ*, or "Skippers," are soluble with difficulty, and do not yield the reactions described.

In the apparently strict confinement of these special pigments to the Pieridæ, we have interesting evidence justifying the customary classification of these insects as a natural group; but the fact has a still more important bearing on the subject of mimicry. The resemblance between the genus *Leptalis* (Pieridæ) and certain of the *Heliconiidæ* is, perhaps, the best known of all instances of protective mimicry. I have found that the mimicking Pierid retains the characteristic pigments of its group, while those of the mimicked Heliconid are quite distinct. This would seem wholly to refute the argument that in such cases the likeness may spring from a real affinity between the two insects.

* I have for instance been quite unable to confirm the observations of A. B. Griffiths, referred to at the commencement of this paper. Soluble green pigments undoubtedly exist in the various species mentioned by this author (*loc. cit.*), but after many efforts I have been unable to obtain from them any uric acid reactions whatsoever.

† 'Entomologist,' 1889-91; 'Nature,' xlv., pp. 513, 514.

Thus, I obtained a supply of *Leptalis* (*vel Dismorphia*) *praxinoë*, and found that the yellow and orange pigments of the wing gave a yellow solution in hot water, which, heated with sulphuric acid, rapidly turned pink and showed the characteristic bands of lepidoporphyrin. A murexide reaction was also easily obtained from the purified substance. On the other hand, various species of *Mechanitis*, including *M. doryssus*, *M. lysidice*, and others, though containing pigments soluble in water, gave solutions which yielded no trace of either lepidoporphyrin or murexide.

IX. *The Pigments in the Chrysalis.*

In the butterfly metabolism is reduced to a minimum, and there is every reason to suppose that the pigmentation of the wing is practically unaffected during the life of the imago. The uric acid and the substances allied to it, which are found on the wings of a Pierid must be formed during the rapid tissue changes which occur in the pupa. A collection of renal excretory products in the rectum during the pupal stage is a common phenomenon in insects, and it would seem as though the Malpighian tubes of the future imago come into action some time before the completion of development. Thus the blow-fly when it first emerges from the pupa-case excretes a mass of nearly pure uric acid. POULTON has shown that among butterflies the Vanessidæ excrete uric acid under like circumstances. I have myself observed that the yellow Pieridæ also void uric acid immediately after they leave the chrysalis, and it is a point of great interest to note that this is associated with a yellow pigment, almost certainly the same as that found in the wings.* I have obtained clear lepidoporphyrin reactions from the yellow material so voided. The fact is thus emphasized that the substances used in ornament are the true excretory products of the animal.

The nature of the process whereby these products reach the wing-scales can only be ascertained by a careful study of pupal development. I have myself repeatedly observed that the scales are fully developed anatomically before the pigments appear in them. No murexide or lepidoporphyrin reactions can be obtained from the embryo wings until the perfect insect is close upon emergence, not, I believe, much before the time when similar excretory products have begun to accumulate in the rectum. As pigments similar to those found in the matured wing-scales of many butterflies, are also present in the hair-like scales which clothe the body, it would seem that the existence of excretory products in the wing is not to be explained by any reference to the ancestral history of the special organ.

The possibility of tracing the process of pigmentation in the developing pupa makes the whole problem one of special attractiveness. My own work in this latter direction is, however, too incomplete to be referred to in this paper.

* Dr. URECH has pointed out that the pigments found in the excreta of butterflies are apt to be of the same colour as those predominant in the wing (BEDDARD'S, 'Animal Coloration,' p. 41).

Summary of the Facts detailed in this Paper.

1. The scales of white Pieridæ contain uric acid, and the presence of this substance is, in fact, the cause of the opaque milky whiteness, characteristic of the wings of such insects.

2. The scales of allied yellow insects are pigmented with a substance closely related to uric acid, the yellow pigment being in all probability identical with a product obtained when uric acid is heated with water under pressure.

3. The yellow substance may be traced in all the genera of the Pieridæ, and with the exception of another closely allied red pigment, is probably the only coloured scale-pigment present; its colour effects may, however, be modified by superadded interference phenomena, or by pigments found, not in the scales, but between the chitinous laminæ of the wing itself.

4. Pigments, giving the reactions described in this paper, are apparently not found in other groups of butterflies, so that when the Pierid mimics an insect belonging to another group the pigments in the two cases may be easily distinguished chemically.

5. The yellow wing pigment is frequently found in material voided from the rectum of a Pierid, showing that in the coloured insects, as well as in the white, a normal excretory product subserves the purpose of ornament.

Most of the insects used in this investigation have been obtained as occasion offered, through the dealers, but I have to thank Professor Meldola, Mr. William Bateson (of St. John's College, Cambridge), and others, for contributions to my store of material. I have also obtained specimens of many rare insects (which have enabled me to trace the presence of lepidotic acid in nearly all the genera of the Pieridæ) from duplicates in the magnificent collection of Mr. Grose-Smith. The greater part of the work was carried out in the laboratories of Guy's Hospital during my tenure of the Gull Research Studentship. I owe much to the care and enthusiasm of Mr. H. C. Coram, who assisted me. Finally, my grateful acknowledgments are due to Professors Meldola and Ray Lankester for much kind encouragement I have received from them.

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES